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Physicochemical and microbial characteristics of gasoline polluted soil in Umuimo municipal in Osisioma L.G.A Abia state.

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Abstract
The physicochemical and microbial properties of gasoline polluted farmland at Umuimo municipality, in Osisioma Local Government Area of Abia state, Nigeria. The investigation was insitu. The impacted farmland has less vegetation growth on it compared to areas around it that was not impacted. The physiochemical and microbial characteristics were determined at interval of 15 space days (15,30,45 days). The control sample's physicochemical average results were: Total Petroleum Hydrocarbon (TPH) 0.08mg/kg, Potassium (K) 10.26mg/kg, Nitrogen (N) 1.27mg/kg, Phosphorous (P) 3.26mg/l, pH 5.9 whereas the polluted farmland were: TPH 216.28mg/kg, K 3.78mg/kg, N 0.46mg/kg, P 1.00mg/kg, pH 6.6. Microorganisms determined in the control sample were Bacteria: Bacillus sp, Corynbacterium sp, Micrococcus whereas the microorganism in the polluted soil sample were Bacillus subtilus, Micrococcus, Corynbacterium, Acinetobacter, Bacillus, Pseudomonas, Spirillum. The microbial count of the control soil were - Total heterotrophic bacteria decreased from 2.76 x 10^5 to 1.64 x 10^5 Colon forming unit per gramme (cfu/g), Total hydrocarbon utilizing bacteria decreased 2.54 x 10^5 to 1.15x10^5 cfu/g. For the polluted soil Total heterotrophic bacteria increased from 1.94x10^6 to 2.15x10^6 cfu/g, Total hydrocarbon utilizing bacteria increased from 1.63x10^6 to 2.01x10^6 cfu/g. The result showed gradual reduction of TPH in the samples as days increased, also the concentrations of Nitrogen, Phosphorous and potassium decreased as days increased.

Keywords: *Gasoline, Physicochemical, Microbial analysis, Hydrocarbons.*

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I. Introduction

Umuimo municipal is in Umueze Autonmous community (along Aba-Owerri expressway) in Osisioma Local Government Area (LGA). Osisioma Ngwa LGA covers about 198Km^2 with coordinates of $5^0$8'59''N, $7^0$19'49''E, and a population of 219,632 (2006 census). It has table topography and its soil profile grades from fine silty sands to fine gravel sand. The general soil characteristic is dominated by silts, sands and sandy clay in different proportions. Osisioma Ngwa shares boundaries with Ukwa West and Umunagbo in the South, Aba South LGA and Aba North LGA in the East, Isiala Ngwa South LGA in the North.

Oil production, transportation and distribution activities have consequences of environmental pollution. Crude and processed petroleum are transported from the producing points to different locations and the activities are prone to leakages, vandalization, explosions and fire outbreak. In 12th October, 2018, a farmland at Umuimo municipality in Osisioma Local Government Area in Abia State, was impacted by a petroleum hydrocarbon due to gasoline pipeline rupture which led to fire outbreak. Many lives, residential buildings, economic trees and vegetation were adversely affected.

Gasoline is hydrocarbon with many components such as inorganic compounds, straight chain, branched chain, cyclic chain. Significant quantity of petroleum products in environment has adverse effect on organisms, including humans (Alexander 1994). Some routes of environmental pollution are through spillage, flow leakage, effluents from anthropogenic activities (Chikere, Chijioke–Osuji 2006). Gaseous components volatilize from the polluted surface and leave the non-volatile components as residues (Odu, 1977). Physical and chemical nature of soil are affected by oil pollution (Minai-Tehran, Herfatmanesh 2007). Some adverse impact of Oil pollution on economic and environment are enormous; damage to vegetation, soil fertility, microorganisms and so on (Nwachukwu, Ugorji 1995). Toxicity of the pollutant varies depending on the type of oil, additives used during processing as well as the biota of spillage (Reddy, 2001).

Fig 1 Cross section of the polluted farmland

Ujowundu, C.O. et al. (2011) study on diesel oil contaminated soil stated that the total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAH) concentrations were 46,726.80mg/kg and 844.40mg/kg respectively whereas TPH and PAH of control soil were 2.90mg/kg and 0.001mg/kg respectively. The result reviewed an increase on heavy metals (Cadmium, iron, lead) and organic carbon and reduction in microbial biomass. These adverse changes could affect nutrient cycle, hinder nutrient uptake by plant roots which will lead to poor agricultural yield.

Eze, V.C. et al.(2014) determined the microbiological and physicochemical characteristics of the soil polluted by generating plant oil around Umuahia metropolis (filling station, mechanic workshop, areas around generating plants). Physicochemical composition of the different soil samples showcased different temperature readings (28.0 - 30.0^{o}C), slightly acidic. The nitrate and phosphates were higher in value than the control sample. The indigenous microorganisms present in the polluted soil were include Bacillus, Micrococcus, Alcaligenes spp., Flavobacterium, Corynebacterium species, and Streptococcus species. Atlas, R.M (1981) stated that neutral pH encourages biodegration. The type of microorganisms that participate in hydrocarbon degradation is determined by the pH of the soil (Bossert, I. & Bartha, R., 1984). Antai, S.P.(1990) in his report stated that Bacillus, Micrococcus, Alcaligenes spp., Flavobacterium, Corynebacterium species, and Streptococcus species are hydrocarbon degrading microorganisms.

II. Methodology

2.1 Sampling of Soil samples

Soil samples were collected from five different locations (surface and subsurface at depth of 15cm) labeled AP, BP, CP, DP, EP at the study site. The sampling points were based on the areas that had evidence of oil. The control sampling was done at about 100m away from the polluted site and were tagged A,B,C,B,E. Sampling were done with stainless knife, 1 litre plastic bottles and they were cleaned by 10% nitric acid and distilled water. All parameters of each sample were measured and their mean values were used for data analysis.

2.2 Physicochemical Analysis

The physicochemical properties of the five samples of soil from the polluted and unpolluted sites were determined. Sampling was done within the space interval of 15days, 30days and 60days. The parameters determined were pH, Nitrogen, Potassium, Phosphorous and Total petroleum hydrocarbon (TPH).

The pH of the samples was determined as follows; twenty grammes of air-dried soil sample was introduced into a 50ml beaker. 20ml of distilled water was added, thoroughly stirred with a glass rod and allowed to stand for 30 minutes. The electrode of the pH meter (Mettler Delta 340) was inserted into the partly settled suspension and the pH measured. (AOAC, 1984)

2.1 **Determination of soil physicochemical properties**

Total Nitrogen was measured by Kjeldahl digestion, Potassium was determined by using a flame photometer and Phosphate was estimated by using procedures of Maiti (2003).

2.2 **Determination of total petroleum hydrocarbons**

Soil sample (2g) and chloroform/dichloromethane (10mls) were vigorously mixed clean extraction container, filtered thereafter with filter paper fitted into Buchner funnels. The concentrated aliphatic fractions were transferred into labeled vials with Teflon caps for gas chromatograph analysis.

2.3 Total Heterotrophic bacteria count.

The pour plate method was engaged. 1 ml of the soil suspension was introduced aseptically into sterile Petri-dishes which were in triplicates and about 15ml of an already sterilized nutrient agar (Oxoid) was poured into each plate and then gently swirled and allowed to cool. The incubation temperature was 300C for 1 – 2 days. Thereafter the colonies formed were counted and expressed in colony forming units.

2.3.1 Total Hydrocarbon degrading bacteria

The pour plate method was used using oil agar medium. 1ml of the soil suspension was introduced aseptically into sterile Petri-plates (in duplicates) and sterilized oil agar was poured aseptically into the plates and gently swirled and were then allowed to gel before they were incubated at 30^0C for 5-7 days. Colonies were seen on the plates and then counted. Hydrocarbon served as the sole carbon source.

2.4 **Isolation and Staining of bacteria**

Bacterial colonies that grew on the oil agar plates were randomly picked based on their morphological differences and sub-cultured by streaking on nutrient agar plates in order to purify the selected colonies. Further sub-culturing was carried out so as to further obtain pure cultures which were preserved in nutrient agar slants and stored. Pure culture of the isolates was Gram stained as described by Collins and Lyne (1984). A thin smear was made on a clean grease-free slide and heat-fix. The smear was then stained with 2 drops of the basic 2% (w/v) crystal violet, the primary stain for 30seconds. This was followed by treatment with 2.5% Gram's Iodine solution functioning as a mordant for one minute. The iodine increases the interaction between the cell and the dye so that the cell is stained more strongly. The smear was next decolorized by washing with 95% ethanol for 10 seconds until no more violet coloration was observed. The slide was rinsed under gently running tap water followed by counter-staining with 2 drops of safranin for 30 - 40 seconds. Dry the film with blotting paper, and observe under oil immersion objectives lens of the microscope. The bacteria that take stain and appear dark violet or blue black are called Gram-positive bacteria. Colonies that developed on nutrient agar plates were grouped on the basis of their colonial morphology.

III. Results and Discussion

Table 3.0 Probable microorganisms present in the soil samples

	Control sample	**Gasolene impacted sample**
1	Bacteria	Bacteria
2	Bacillus sp	Bacillus subtilus
3	Corynbacterium sp	Micrococcus
4	Micrococcus	
5		Corynbactrium
6		Acinetobacter
7		Bacillus
8		Pseudomonas
		Spirillum

Table 3.1 Mean values of the microbial count of control and impacted soil samples

Days	Microorganisms	control soil(cfu/g)	Impacted soil(cfu/g)
15days	Total Heterotrophic bacteria	0.276×10^6	1.94×10^6
	Total Hydrocarbon degrading bacteria	0.254×10^6	1.63×10^6
30days			
	Total Heterotrophic bacteria	0.185×10^6	2.08×10^6
	Total Hydrocarbon degrading bacteria	0.118×10^6	1.81×10^6
45 days			
	Total Heterotrophic bacteria	0.164×10^6	2.15×10^6
	Total Hydrocarbon degrading bacteria	0.115×10^6	2.01×10^6

Table 3.2 Physico-chemical characteristics of control soil samples

No	**Parameters**	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**
Duration 0-15 days						
1	TPH (mg/kg)	0.08	0.08	0.05	0.076	0.08
2	K (mg/kg)	10.89	10.54	10.89	10.95	11.01
3	N(mg/kg)	1.56	1.52	1.49	1.56	1.50
4	P(mg/kg)	4.3	3.9	4.10	4.3	3.94
5	pH	5.8	5.8	6.1	5.9	6.1
Duration 16-30days						
1	TPH (mg/kg)	0.06	0.07	0.05	0.070	0.08
2	K (mg/kg)	10.89	10.50	10.81	10.93	10.91
3	N(mg/kg)	1.32	1.50	1.43	1.34	1.41
4	P(mg/kg)	4.01	3.88	3.94	4.01	3.89
5	pH	5.8	5.8	6.1	5.9	6.1
Duration 31 - 45 days						
1	TPH (mg/kg)	0.05	0.060	0.03	0.05	0.06
2	K (mg/kg)	7.98	9.01	8.21	10.15	10.18
3	N(mg/kg)	0.98	0.88	0.76	0.91	0.94
4	P(mg/kg)	1.93	2.01	1.58	2.04	1.07
5	pH	5.8	5.8	6.1	5.9	6.1

Table 3.3 Mean value of parameters in the control soil samples

Duration	TPH	K	N	P	pH
15days	0.07	10.86	1.53	4.11	5.9
30 days	0.07	10.81	1.4	3.95	5.9
45 days	0.05	9.11	0.89	1.73	5.9

Total	0.19	30.78	3.82	9.79	17.7
Mean	0.06	10.26	1.27	3.26	5.9

Table 3.4 Physico-chemical characteristics of impacted soil samples

No	Parameters	Sample AP	Sample BP	Sample CP	Sample DP	Sample EP
Duration 0-15 days						
1	TPH (mg/kg)	216.28	228.00	218.43	231.15	238.01
2	K (mg/kg)	5.74	5.21	6.42	6.01	5.51
3	N(mg/kg)	0.65	0.71	0.68	0.82	0.85
4	P(mg/kg)	1.86	1.90	2.10	2.26	1.55
5	pH	6.8	6.5	6.5	6.8	6.6
Duration 16-30days						
1	TPH (mg/kg)	194.05	183.21	169.24	150.89	177.32
2	K (mg/kg)	3.8	2.98	4.01	3.99	3.45
3	N(mg/kg)	0.40	0.52	0.38	0.43	0.51
4	P(mg/kg)	0.86	0.68	0.88	0.78	0.49
5	pH	6.8	6.5	6.5	6.8	6.6
Duration 31-45days						
1	TPH (mg/kg)	101.21	98.12	84.56	75.01	77.7
2	K (mg/kg)	2.4	1.84	2.04	1.64	1.57
3	N(mg/kg)	0.28	0.15	0.11	0.13	0.21
4	P(mg/kg)	0.54	0.22	0.38	0.35	0.12
5	pH	6.8	6.5	6.5	6.8	6.6

Table 3.5 Mean Value of the parameters of the impacted soil samples

Duration	TPH	K	N	P	pH
15days	226.37	5.78	0.74	1.95	6.64
30 days	174.94	3.65	0.45	0.74	6.64
45days	87.32	1.90	0.18	0.32	6.64
Total	488.63	11.33	1.37	3.01	19.92
Mean	162.88	3.78	0.46	1.00	6.64

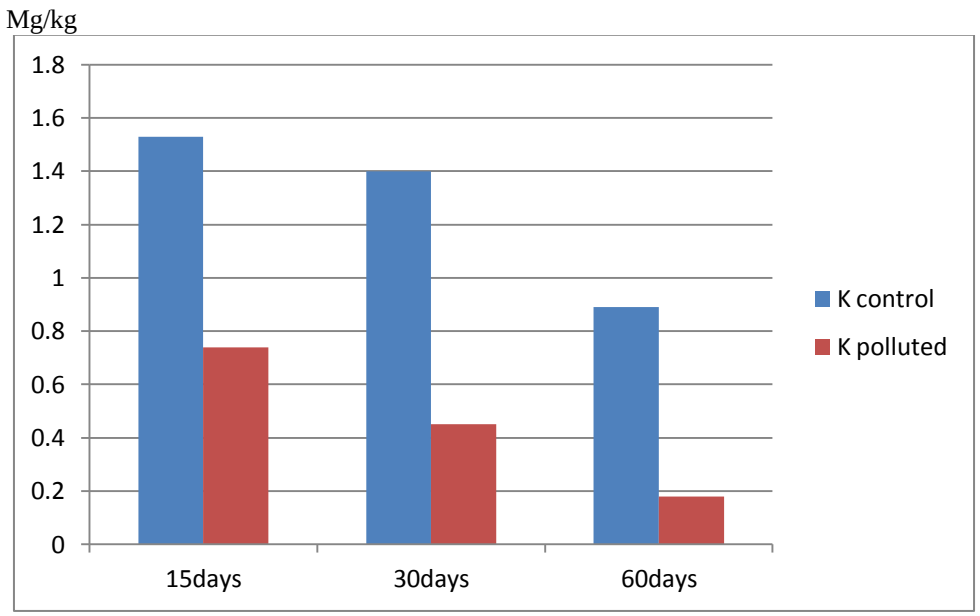

Fig 3.1 Mean Concentration of potassium in control sample Vs Impacted sample

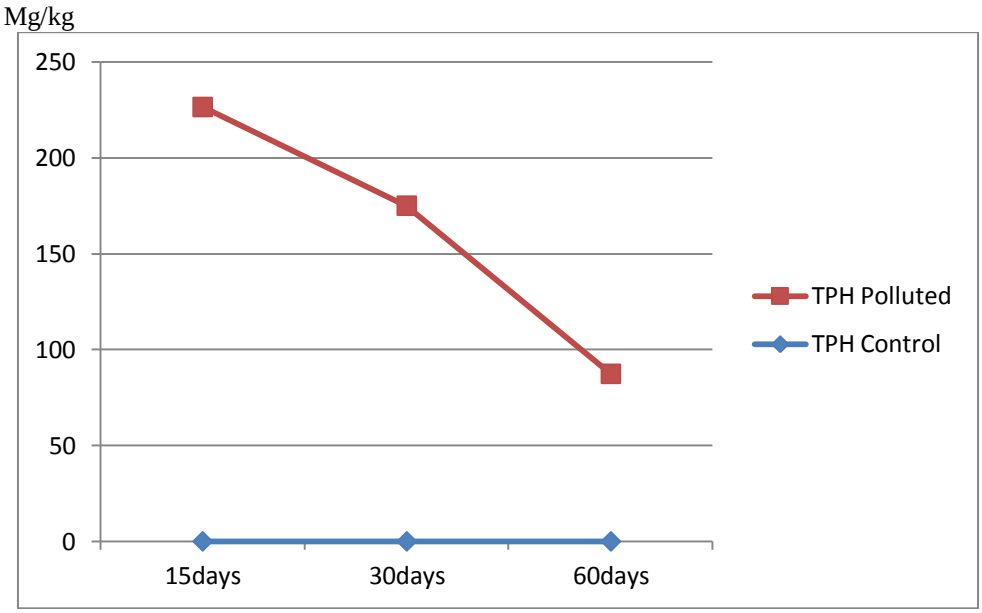

Fig 3.2 Mean concentration of TPH in control soil Vs impacted soil.

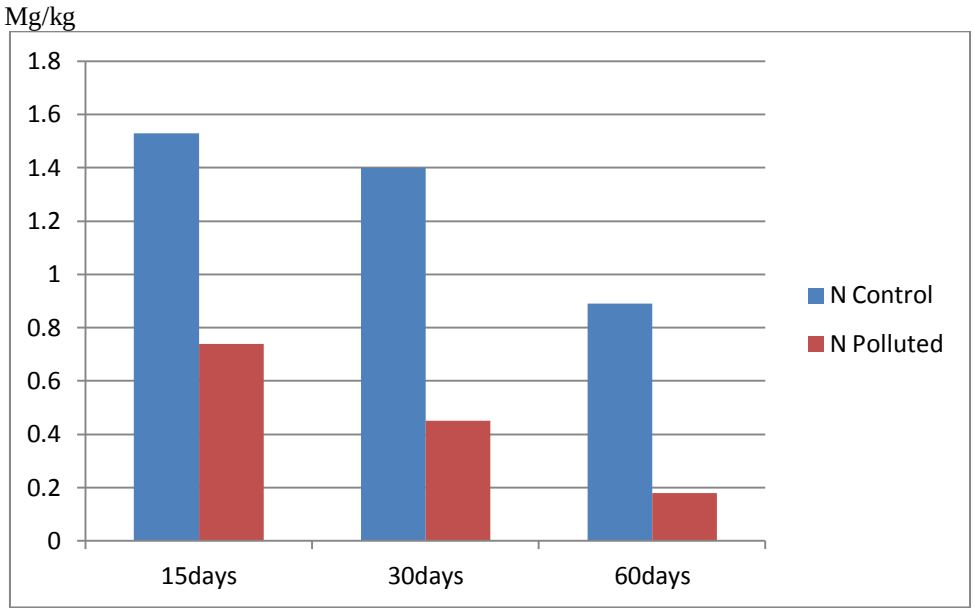

Fig 3.3 Mean concentration of Nitrogen in control soil Vs impacted soil

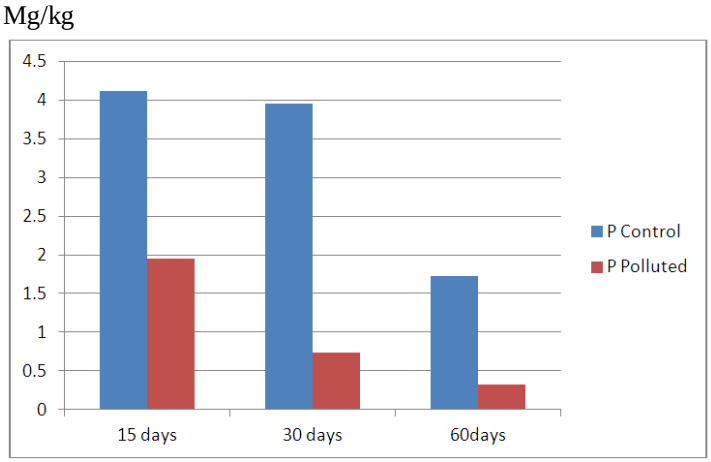

Fig 3.4 Mean concentration of Phosphorous in Control soil Vs Impacted soil

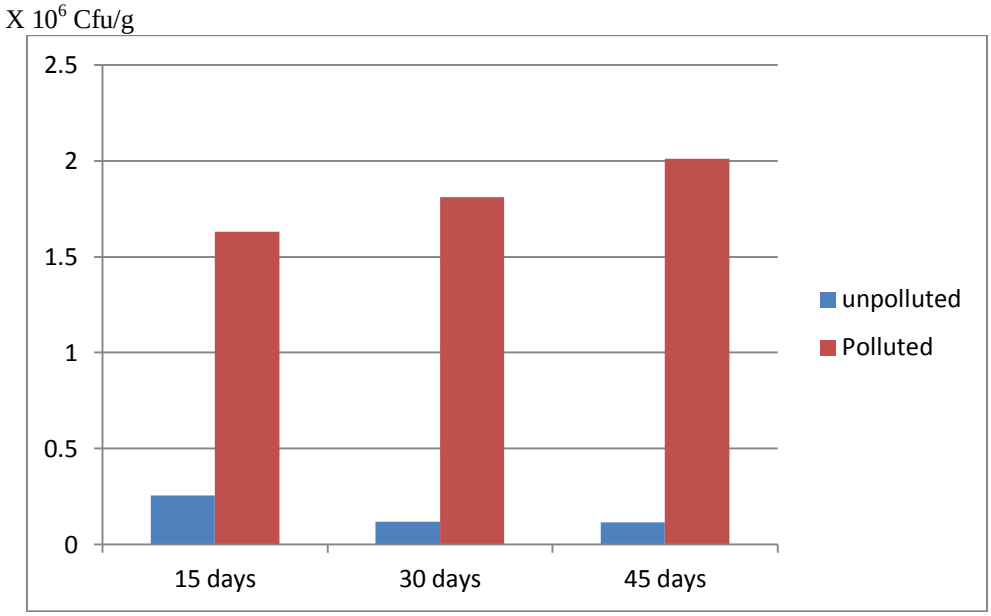

Fig 3.5 Total heterotrophic bacteria in control soil vs Impacted soil

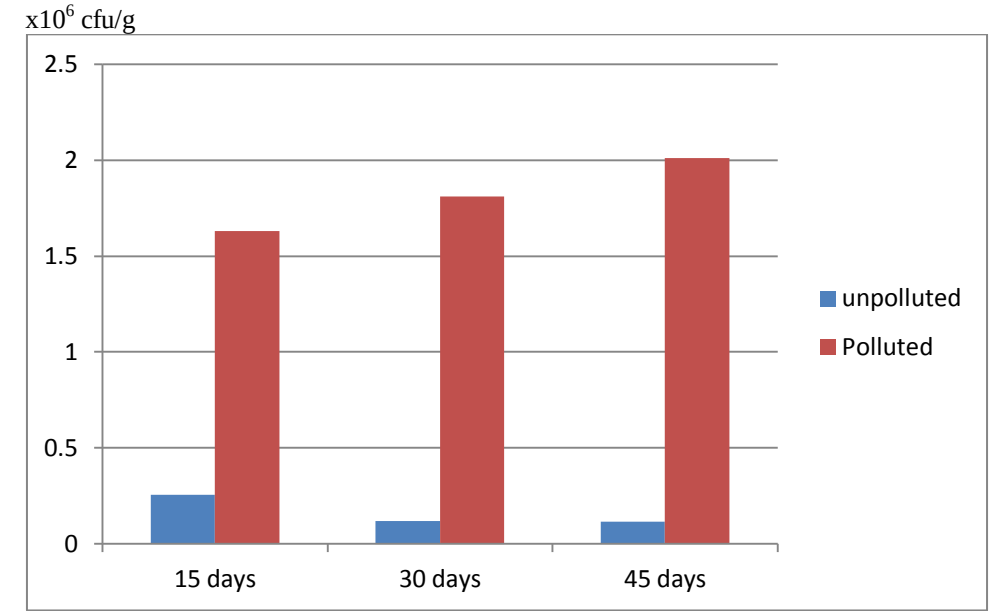

Fig 3.6 Total hydrocarbon degrading bacteria in control soil vs impacted soil

The present investigation has revealed the differences in soil characteristics (physico-chemical and microbial analysis of the soils) as influenced by the presence of refined petroleum hydrocarbons (Gasolene). The results of the physicochemical parameters for the control sample and the gasoline impacted samples are shown in Tables 3.3 and 3.5 respectively.

At the end of the investigation, the mean values of potassium, nitrogen and phosphorous in the control soil were 10.26mg/kg, 1.27mg/kg, 3.26 mg/kg respectively while the values of potassium, nitrogen and phosphorous in the gasolene impacted soil were 3.78mg/kg, 0.46 mg/kg and 1.00mg/kg respectively. It showed a decreasing trend of the nutrient. The initial value of TPH of the polluted soil was 226.37mg/kg and at the end of the investigation (45 days), TPH dropped to 87.32mg/kg. This result is in conformity with Amadi et al (1993) result which stated a decrease in the quantity of TPH and nutrients in the soil impacted with petroleum hydrocarbon at the end of their investigation.The decrease in TPH and nutrients might be the presence of microorganism in the soil. Microorganism feed on the nutrients and hydrocarbons for growth. This result was in agreement with the result of Agarry et.al experiment which reported that 73% and 50% TPH loss for hydrocarbon polluted soil treated with poultry manure and goat manure respectively.

The mean pH of the hydrocarbon impacted soil was about 6.64 while that of control soil was 5.9. It showed that the pH of the impacted soil tended to neutrality. Table 3.1 shows that at the end of the investigation

the total heterotrophic bacteria and total hydrocarbon utilizing bacteria in control soil were $0.164x10^6$ cfu/g and $0.115\ x10^6$cfu/g respectively while the impacted soil had $2.15\ x\ 10^6$cfu/g and $2.01x10^6$cfu/g of total heterotrophic bacteria and total hydrocarbon degrading bacteria respectively. The result is similar to Atlas (1981) result which reported that neutral pH support growth of microorganism which boosts biodegradation activity of bacteria.

IV. Conclusion

The impart of gasoline farmland was investigated. Physiochemical properties had it that the nutrients (N, K, P) and TPH in the polluted soil diminished gradually as days go on. It was established that microorganisms might have fed on the petroleum hydrocarbon and nutrients which might lead to increase in their numerical strength.

The microbial analysis of the polluted soil showed increase in microorganisms population. It was concluded that hydrocarbon polluted soil attracts microorganisms natural especially the hydrocarbon degraders. At the end of the investigation, the heterotrophic bacterial species isolated from control soil were Bacillus sp, Corynbacterium sp, Micrococcus, Klebsella , while the gasolene polluted soils had Bacillus subtiles, Micrococcus, Corynbacteria sp, Pseudomonas, Spirillum. the total heterotrophic bacteria and total hydrocarbon utilizing bacteria in control soil were $0.164x10^6$ cfu/g and $0.115\ x10^6$cfu/g respectively while the polluted soil had $2.15\ x\ 10^6$cfu/g and $2.01x10^6$cfu/g of total heterotrophic bacteria and total hydrocarbon degrading bacteria respectively. The were more species of microorganisms in the impacted soil than in the control soil, also the microbial count was higher in the impacted soil than in the control soil. This might be due to presence of hydrocarbon in the polluted soil. This result is in conformity with Amadi and Odu (1993) investigation which reported an initial gradual increase in bacterial population following the application of petroleum hydrocarbon but a decline as the biodegradation progressed.

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